

## TEMPERATURE SHIFT EFFECTS ON INJURY AND DEATH IN *LISTERIA MONOCYTOGENES* SCOTT A

### ABSTRACT

*Exposure of Listeria monocytogenes Scott A grown at 37°C to a 1 h heat treatment at 52°C resulted in little death of the cells (< 0.5 log). However, as the temperature of growth decreased, there was an increase in the extent of death (> 4 logs at 10°C growth temperature). Heat induced injury, however, decreased as the growth temperature decreased. Shifting L. monocytogenes grown at 10, 19, or 28°C to 37°C for periods up to 5 h led to cells with increased heat tolerance. However, there was little effect on injury by the shift-up procedure. Presence of chloramphenicol during the shift-up period inhibited the gain in heat tolerance. L. monocytogenes grown at low temperatures ( $\leq 28^\circ\text{C}$ ), were more susceptible to killing by heat, but this susceptibility could be lost if cells grown at low temperatures are given a short incubation at 37°C. The data obtained here suggest that if foods containing L. monocytogenes are temperature-abused for even short periods, the organisms will acquire an increased heat tolerance and will require higher inactivation temperatures or longer processing times.*

### INTRODUCTION

A number of factors affect the heat resistance of microorganisms including the nature and concentration of the ingredients present in the culture medium, pH of the medium, physiological status and age of the cells, and the temperature at which the cells are grown (Hansen and Riemann 1963; Beuchat 1978). One of the more interesting of these factors is the influence of growth temperature on thermal tolerance.

Growing *Escherichia coli* at temperatures ranging from 28 to 40°C, Elliker and Frazier (1938) demonstrated that when cells grown at the lower temperatures were subjected to a 53°C heat stress, they were less heat resistant. Similarly, *Salmonella seftenberg* 775W, *S. typhimurium*, *Vibrio parahaemolyticus* and *Streptococcus faecalis* were much more susceptible to heat inactivation if they were grown at low temperatures (Ng *et al.* 1969; Dega *et al.* 1972; Beuchat and Worthington 1976; White 1953). Knabel *et al.* (1990) found that growing *Listeria monocytogenes* at temperatures higher than 37°C (39 to 43°C) produced cells that were more heat resistant than 37°C grown cells. Thus, growth at low temperatures appears to produce microbial cells which are more sensitive to heat destruction.

In this study, we used *L. monocytogenes*, Scott A strain, as a representative strain of the species to determine the effect of low growth temperature on heat destruction and injury of the organism as well as to determine the effect on heat tolerance of a shift-up from low growth temperatures to 37°C.

## METHODS AND MATERIALS

### Microorganism

*L. monocytogenes*, Scott A strain, was maintained at 5°C in brain heart infusion broth (BHI; Difco). Fifty mL of BHI containing additional glucose (BHIG; final glucose concentration, 0.5% w/v) was inoculated and then incubated on a rotary shaker (150 rpm) at 28°C for 22–24 h. An aliquot of the 28°C grown cells was inoculated into 50 mL BHIG contained in 500 mL flasks and incubated shaking at temperatures ranging from 10 to 42°C. Cells, at maximum stationary stage, were harvested by centrifugation, washed twice with sterile distilled water and resuspended in 5 mL sterile distilled water. For temperature shift studies, cells grown at 10, 19, or 28°C to stationary phase were incubated at 37°C for varying time periods before harvesting. In some of the shift-up experiments, 50 or 100 µg/mL chloramphenicol (Sigma) or ampicillin (Sigma) was added to the growth flasks just before transferring them to 37°C. The harvesting step for antibiotic-treated cells was similar to that of untreated cells.

### Injury Procedure

To determine heat injury, screw-cap 160 mL glass milk dilution bottles containing 20 mL sterile potassium phosphate buffer (pH 7.2, 0.1 M) were equilibrated to 52°C in a water bath and then 1.0 mL of washed *L. monocytogenes* was added to give  $2-4 \times 10^9$  cells/mL buffer. The buffer-cell suspension was agitated (approximately 150 agitations/min) on a Burrell Wrist-Action Shaker. At zero time and at 1 h, 0.1 mL of cell suspension was removed from each bottle and added to 9.9 mL sterile 0.1% (w/v) Bacto peptone water tubes and

successive dilutions prepared. Using a spiral plater (Spiral Systems Instruments, Inc., Bethesda, MD), appropriate dilutions were plated onto tryptose phosphate broth + 2% (w/v) agar (TPBA) + 1% (w/v) sodium pyruvate (TPBAP) and TPBA + 5% (w/v) added NaCl (TPBAS). Plates were incubated at 37°C and counted after 3 days.

Death and injury were monitored by the use of a differential plating technique consisting of TPBAP and TPBAS. Both noninjured cells and injured-repaired cells form colonies on TPBAP whereas only noninjured cells form colonies on TPBAS; repair of injured cells does not take place in the presence of sodium chloride (Smith 1990). Increase in death was defined as number of survivors  $TPBAP_{t=0}$  minus number of survivors  $TPBAP_{t=1}$ . Increase in injury was defined as number of survivors  $TPBAS_{t=0}$  minus number of survivors  $TPBAS_{t=1}$  minus increase in death.

### Statistics

Data were analyzed by one-way analysis of variance using the Ecstatic (Somewhere in Vermont, Montpelier, VT) and Number Crunching Statistical System (J. L. Hintze, Kaysville, UT) statistical software programs.

## RESULTS

As the temperature of growth decreased from 37°C to 10°C, there was an approximate 4 log increase in death to *L. monocytogenes* subjected to 52°C for 1 h (Table 1). However, there was a gradual decrease in the extent of heat induced injury as the growth temperature decreased. Shifting *Listeria* from low temperature growth conditions to 37°C for short periods led to increased thermal tolerance of the cells (Table 1).

*L. monocytogenes*, grown at 10 or 19°C, showed a marked decrease in killing when shifted to 37°C for 5 h before being subjected to 52°C heat treatment. There was only a slight decrease in killing when cells grown at 28°C were shifted to 37°C. For cells grown at 19°C, a shift-up time at 37°C for 1, 2.5, and 5 h gave an 18, 79, and 117-fold increase, respectively, in survivors upon heating at 52°C (Table 1). While shifting *L. monocytogenes* from a lower temperature to 37°C led to a decrease in the extent of death, there was not any great effect on injury. In order to show if protein synthesis might be involved in the shift-up protection against heat killing, chloramphenicol was added to 19°C grown cells just prior to shifting to 37°C. The data presented in Fig. 1 indicates that chloramphenicol inhibited the shift-up increase in tolerance to heat. There was an approximate 75-fold decrease in the number of surviving *L. monocytogenes* when a 100 µg/mL chloramphenicol was present during the 5 h shift-up period whereas 100 µg/mL ampicillin was less inhibitory, with only a 9-fold decrease.

TABLE 1.  
EFFECT OF GROWTH TEMPERATURE AND SHIFT-UP IN TEMPERATURE ON DEATH AND INJURY IN *LISTERIA MONOCYTOGENES*.

growth temperature	conditions	n <sup>a</sup>	Log <sub>10</sub> cfu/ml at zero time		Log <sub>10</sub> cfu/ml after 1 h at 52 °C		death <sup>b</sup>	injury
			TPBAP	TPBAS	TPBAP	TPBAS		
37 °C	no change	29	9.45 (0.14) <sup>c</sup>	9.38 (0.15)	9.05 (0.31)	6.68 (0.61)	0.39 (0.27) <sup>1</sup>	2.31 (0.59) <sup>3</sup>
28 °C	no change	8	9.59 (0.08)	9.51 (0.07)	6.78 (0.34)	3.72 (0.62)	2.81 (0.31) <sup>6</sup>	2.98 (0.53) <sup>5</sup>
28 °C	shift to 37-5h	8	9.67 (0.08)	9.58 (0.11)	7.45 (0.43)	4.36 (0.76)	2.22 (0.48) <sup>4</sup>	3.01 (0.72) <sup>5</sup>
19 °C	no change	22	9.62 (0.13)	9.54 (0.18)	5.96 (0.66)	3.72 (0.71)	3.64 (0.63) <sup>7</sup>	2.22 (0.77) <sup>3</sup>
19 °C	shift to 37-1h	8	9.43 (0.33)	9.39 (0.40)	7.02 (0.40)	4.90 (0.80)	2.39 (0.45) <sup>5</sup>	2.09 (0.68) <sup>2</sup>
19 °C	shift to 37-2.5h	12	9.60 (0.23)	9.59 (0.23)	7.86 (0.52)	5.29 (0.65)	1.74 (0.55) <sup>2</sup>	2.56 (0.62) <sup>4</sup>
19 °C	shift to 37-5h	10	9.62 (0.13)	9.63 (0.07)	8.06 (0.34)	5.59 (0.84)	1.57 (0.44) <sup>2</sup>	2.47 (0.57) <sup>4</sup>
10 °C	no change	8	9.52 (0.09)	9.46 (0.11)	5.25 (0.54)	3.69 (0.62)	4.27 (0.53) <sup>8</sup>	1.50 (0.45) <sup>1</sup>
10 °C	shift to 37-5h	10	9.60 (0.06)	9.56 (0.08)	7.65 (0.42)	5.33 (0.63)	1.95 (0.42) <sup>3</sup>	2.28 (0.66) <sup>3</sup>

<sup>a</sup>n is the number of replicate flasks.

<sup>b</sup>Means in the same column followed by the same superscript number are not significantly different ( $p < 0.05$ ; Fisher's LSD Comparison).

<sup>c</sup>Numbers in parentheses are standard deviation of means.

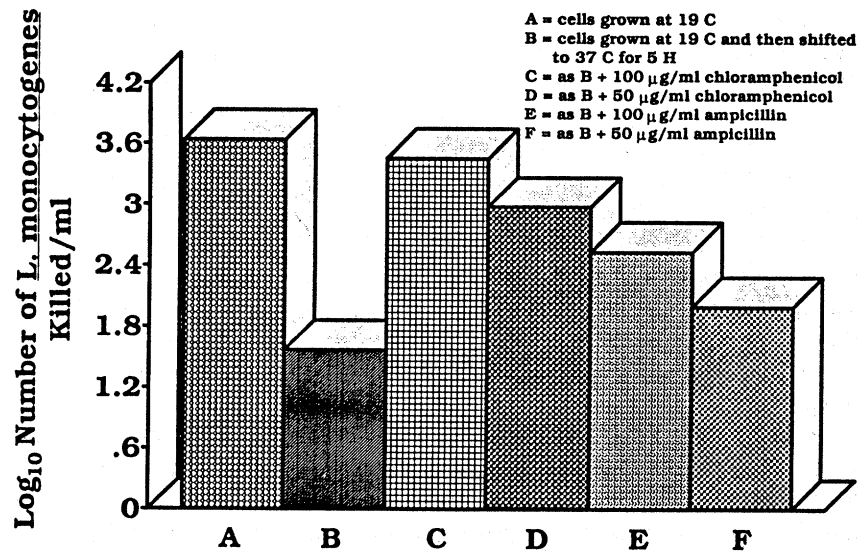


FIG. 1. EFFECT OF CHLORAMPHENICOL AND AMPICILLIN ON HEAT-INDUCED DEATH DUE TO SHIFTING 19°C GROWN *LISTERIA MONOCYTOGENES* TO 37°C FOR 5 H BEFORE HEATING CELLS AT 52°C  
Each bar represents the means of 3 determinations.

## DISCUSSION

The data presented here indicate that growth of *L. monocytogenes* at low temperatures ( $\leq 28^{\circ}\text{C}$ ) yields cells with decreased thermotolerance. Shift of such cells to  $37^{\circ}\text{C}$  for periods as short as 1 h lead to increased heat resistance. Since chloramphenicol inhibits the shift-up increase in heat resistance, protein synthesis, at least in part, may be necessary during the shift-up incubation period for increased thermal tolerance.

A number of microorganisms have been shown to produce heat sensitive cells when grown at low temperatures (Elliker and Frazier 1938; White 1953; Ng *et al.* 1969; Dega *et al.* 1972; Beuchat and Worthington 1976). A few studies have been done demonstrating that shift-up of cells grown at a lower temperature to a higher incubation temperature results in microorganisms that are more heat resistant. Most of these experiments involved shifting  $37^{\circ}\text{C}$  grown cells to temperatures of 42 to  $48^{\circ}\text{C}$ . *Salmonella typhimurium* grown in broth at  $37^{\circ}\text{C}$  and then incubated at 42, 45, or  $48^{\circ}\text{C}$  for 30 min before heat treatment at 50 to  $59^{\circ}\text{C}$  were more thermoresistant than cells not preincubated at the higher temperatures (Mackey and Derrick 1986). Similarly,  $37^{\circ}\text{C}$  grown *S. thompson* inoculated into liquid whole egg, 10 and 40% milk solids, and minced beef followed by incu-

bation at 48°C were more resistant to a 54°C heat treatment than were the organisms present in the untreated foods (Mackey and Derrick 1987). Using 37°C grown *L. monocytogenes* inoculated into cured meat and held at 48°C for 30 min led to cells with increased resistance to heating at 65°C (Farber 1989). When raw sausage mix containing *L. monocytogenes* maintained at 4°C overnight was subjected to heat shock at 48°C for 2 h, there was a significant increase in heat tolerance, i.e., the  $D_{64^\circ\text{C}}$  value increased from 3.3 to 8.0 min (Farber and Brown 1990). UHT milk containing cells of *L. monocytogenes* grown at 37°C in broth followed by incubation at 48°C for 1 h resulted in cells that survived heating at 60°C (Fedio and Jackson 1989); also, there were fewer injured cells. Thus, the protective effect against heat injury and death due to a short incubation at temperatures higher than the growth temperature is not a phenomenon limited to bacterial media but is found with food products, also.

When cells, both eucaryotic and procaryotic, are exposed to sublethal temperature or other types of sublethal stress, small highly conserved polypeptides, the heat shock proteins, are synthesized (Lindquist 1986). The function of the heat shock proteins appear to be protective, i.e., they protect cells against stress damage and may play a role in returning the cells to their normal physiological state following the stressful event (Schlesinger 1988).

Yamamori and Yura (1982) showed that heat shock proteins were produced when *Escherichia coli* K-12 grown at 30°C were shifted to 42°C. Such cells were more resistant to heating at 55°C. The acquisition of heat resistance was inhibited if chloramphenicol was added just before shifting the cells to 42°C. The authors suggested that protein synthesis is involved in the increase in thermotolerance given by the shift-up in temperature and that the protective effect is due to the presence of the heat shock proteins. The thermophile, *Sulfolobus*, demonstrated thermal resistance if 70°C grown cells were exposed to 88°C for 1 to 4 h before subjecting them to the lethal stress of 92°C (Trent *et al.* 1990). Heat resistance was correlated with the appearance of certain proteins which, however, did not appear to be similar to typical heat shock proteins.

Heat shock proteins are produced by 37°C grown *L. monocytogenes* incubated at 48°C for 30 min (Sokolovic and Goebel 1989). However, the role of these proteins in protecting *Listeria* from heat or other stresses has not been studied. In the present study, thermotolerance was obtained by shifting 10 to 28°C grown *L. monocytogenes* to 37°C and incubating for periods up to 5 h. However, 37°C is not normally considered to be a temperature for induction of heat shock proteins. The data presented in Table 1 indicates that a substance, responsible for increased thermotolerance in *Listeria*, is synthesized at  $\geq 37^\circ\text{C}$  which is not synthesized or is synthesized in smaller amounts at lower temperatures. Shifting of *L. monocytogenes* grown at low temperatures to 37°C for short periods resulted in cells with increased tolerance to a 52°C heat treatment. Since chloramphenicol

inhibited the shift-up effect, it is possible that the substance that gives increased thermotolerance to the cells is polypeptide in nature.

The data presented has implications for microbial food safety of foods. The destruction of *L. monocytogenes* by heat depended on the growth temperature. Thus, foods containing *Listeria* held at low temperatures should contain cells that are more sensitive to heat destruction and the foods need only minimal heat processing. However, if such foods are temperature abused for even short periods prior to thermal processing, the cells may become more heat resistant and minimal heat processing would no longer be adequate. This indicates that such foods should be kept refrigerated continuously up to the time of thermal processing.

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